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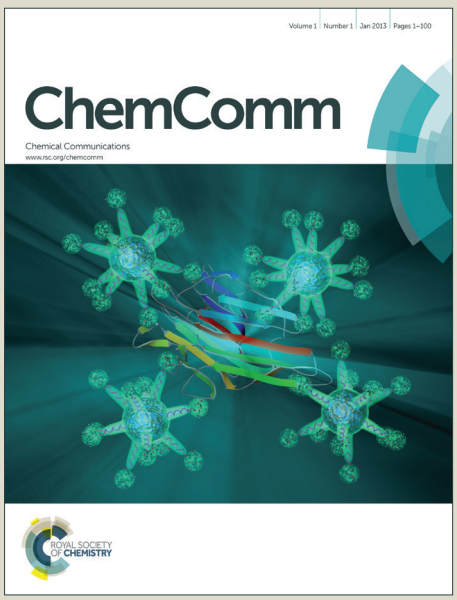

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ARTICLE TYPE

Dendrimersomes with Photodegradable Membranes for Triggered Release of Hydrophilic and Hydrophobic Cargo

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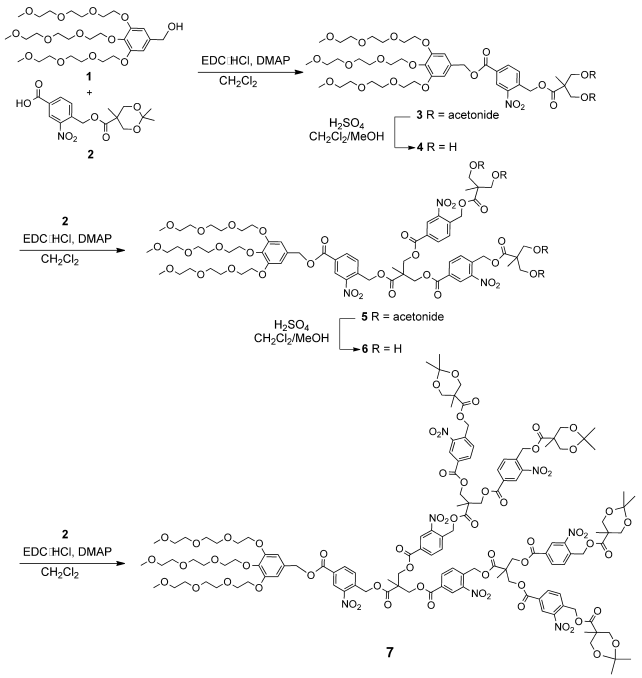
Amphiphilic Janus dendrimers containing first through third generation (G1 - G3) photodegradable hydrophobic blocks were synthesized and their self-assembly in water was studied. While the G1 and G2 systems formed solid aggregates, the G3 system self-assembled to form dendrimersomes. These dendrimersomes were demonstrated to degrade upon irradiation with UV light, and exhibited triggered release of both hydrophobic and hydrophilic payloads.

The step-wise synthesis of dendrimers affords a high degree of control over the positioning of chemical functionalities at the nanoscale.[1] Amphiphilic Janus dendrimers, comprising both hydrophilic and hydrophobic dendrons within the same molecule exemplify this feature, as both the chemical structures and generations of each dendron can be precisely and reproducibly tuned.[2,3] To date, a wide variety of dendritic backbones have been investigated in this context including polyamidoamines,[4-6] polyesters,[7-16] amino acid-based polyamides,[17,18] aliphatic polyethers,[19-21] as well as dendrons with peripheral oligo(ethylene glycol) chains[22,23] and carbohydrates.[24,25] Percec and coworkers recently synthesized more than 100 amphiphilic Janus dendrimers and studied their self-assembly in aqueous media.[14,15] They found that through tuning the chemical structures and dendrimer generations, it was possible to achieve a variety of morphologies including vesicles, which they termed "dendrimersomes". Like their macromolecular analogues polymersomes, dendrimersomes are of significant interest for applications as delivery vehicles[26] or nanoreactors[27,28] as they can simultaneously encapsulate both hydrophilic and hydrophobic species, while also affording a high degree of reproducibility and predictability that is generally not possible with polymersomes.[14] For example, it was recently demonstrated that it was possible to predict the dimensions of dendrimersomes based on their primary chemical structures.[23]

The introduction of stimuli-responsive, and in particular "triggered release" behavior to polymer assemblies and other nanomaterials has attracted considerable attention in the past few decades. Materials responsive to a wide variety of stimuli such as light,[29] mechanical force,[30] and changes in pH[31] and temperature[32] have been developed. Among these stimuli, light is of particular interest for the development of smart materials as it can be applied at a specific time and location with control over its intensity and wavelength. To the best of our knowledge, there are no previously reported examples of photodegradable dendrimersomes. Several different photo-responsive polymersome systems have been developed.[33-39] However, these systems involve either azobenzene moieties that undergo *trans-cis* photoisomerization, or the placement of the photodegradable moiety at the junction between the hydrophilic and hydrophobic blocks of the copolymer. With the exception of a recent example involving self-immolative polymers that depolymerize in the response to UV light,[39] this generally results in residual hydrophobic or amphiphilic materials that can either aggregate to form macroscopic precipitates or undergo rearrangement to other morphologies.[40]

We have recently reported the synthesis of polyester dendrimers with photodegradable *o*-nitrobenzyl units throughout their backbones.[41] It was demonstrated that they undergo complete photolytic backbone cleavage without producing any macromolecular byproducts. Herein, we report the incorporation of these photodegradable dendrons into amphiphilic Janus dendrimers, their self-assembly to dendrimersomes, and their photodegradation. It is demonstrated that UV light can trigger the release of both hydrophilic and hydrophobic cargo.

As shown in Scheme 1, the synthetic strategy involved the divergent growth of the photodegradable polyester dendron from the focal point of the hydrophilic dendritic block. For the hydrophilic block, a backbone based on tri(ethylene glycol) (TEG) and gallic acid, **1**, was synthesized as previously reported.[42] The benzylic hydroxyl focal point was then reacted with our previously reported photodegradable monomer **2**[41] using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC·HCl) in the presence of 4-(dimethylamino)pyridine (DMAP) to afford the protected generation one (G1) dendrimer **3** in high yield. The acetonide protecting groups were then removed under acidic conditions to obtain the deprotected G1 dendrimer **4**. Repetition of this coupling and deprotection sequence provided the G2 and G3 dendrimers **5** - **7**. The molecules were fully characterized by ^{1}H and ^{13}C NMR spectroscopy, high resolution mass spectrometry, IR spectroscopy, and size exclusion chromatography. The data were consistent with the proposed structures and with the very narrow or monodisperse molecular weight distribution expected for dendrimers (ESI).



Scheme 1 Synthesis of photodegradable amphiphilic Janus dendrimers.

The self-assembly of dendrimers **3**, **5**, and **7** was investigated using nanoprecipitation procedures. THF/water and DMSO/water were used as solvent combinations and both orders of addition (organic solvent into water and vice versa) were investigated. The organic solvent was subsequently removed by dialysis. The assemblies were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). In all cases, G1 dendrimer **3** and G2 dendrimer **5** self-assembled to form solid particles, likely micellar aggregates, which have diameters greater than 100 nm as measured by DLS (Fig. 1a and ESI), and which appear as the large grey objects in TEM (Fig. 1b and ESI). It is proposed that although the hydrophilic weight fractions of **3** (0.64) and **5** (0.38) may be suitable for the formation of micelles, their architecture is unfavorable for the formation of stable micelles due to their bulky hydrophobic blocks, which results in their instability and thus aggregation. On the other hand, under all conditions investigated, the G3 dendrimer **7** with a hydrophilic weight fraction of 0.21 appeared to form dendrimersomes. The procedure involving the addition of water to a solution of **7** in DMSO resulted in assemblies with a Z-average diameter of 158 nm and a narrow PDI of 0.02 (Fig. 1c,d). The addition of a DMSO solution of **7** into water resulted in smaller vesicles, while the use of THF/water led to larger vesicles (ESI). An emulsion procedure involving CH_2Cl_2/water, followed by CH_2Cl_2 evaporation, resulted in vesicles with diameters greater than 500 nm that appeared to collapse upon drying (ESI). Overall, these results suggest that the sizes of the vesicles can be tuned by their rate of formation, either through the use of solvents with different polarities or through the rate of solvent change from organic to aqueous.

Given the multifunctional potential of vesicles resulting from the presence of both hydrophilic (core) and hydrophobic (membrane) compartments, photodegradation studies focused on the dendrimersomes formed from **7** (Fig. 1b,c). First, the photodegradation of **7** was studied by UV-visible spectroscopy in THF (~30 μg/mL), a common solvent for both components of the dendrimer. As shown in Fig. 2a, upon irradiation with UV light, over a period of 30 min there were decreases in the intensities of the peaks at 225 and 265 nm, corresponding to the *o*-nitrobenzyl moieties and increases in the peaks at 300 and 340 nm, corresponding to the expected *o*-nitrosobenzaldehyde photolysis product. This was in accordance with the previous observations of our group and others.[41,43] Similar results were observed upon UV irradiation of the dendrimersomes in water, but some background scattering from the assemblies was also observed, complicating the spectra (ESI).

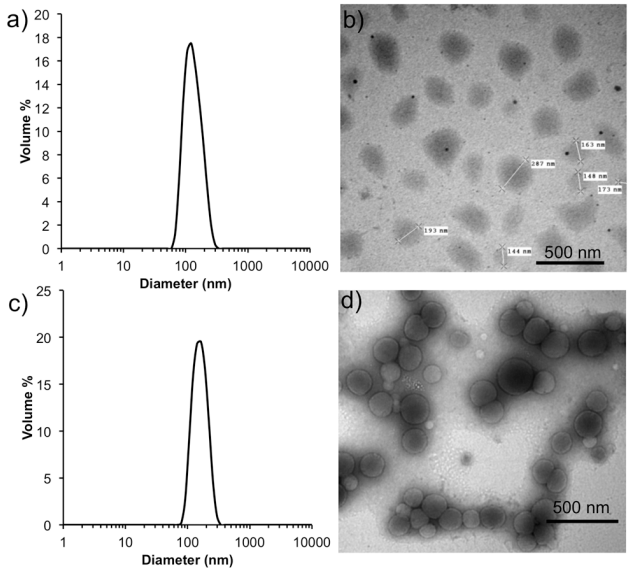


Fig. 1 Selected DLS size distributions and TEM images of the assemblies: a) DLS volume distribution and b) TEM image of assemblies formed from **5**; c) DLS volume distribution and d) TEM image of assemblies formed from **7**. Assemblies were formed by the addition of water into a DMSO solution of the dendrimer. Additional DLS data and TEM images are included in the ESI.

DLS was also used to study the photodegradation of the dendrimersomes. An aqueous suspension of dendrimersomes (0.1 mg/mL) was placed in a quartz cuvette, irradiated with UV light for a total time of 8 h, and DLS measurements were performed in the same cuvette at various time points. As shown in Fig. 2b, over 7 h, the mean count rate decreased from 340 kilocounts per second (kcps) to less than 20 kcps, only 5% of its initial value. Meanwhile the diameters of the assemblies decreased from about 160 nm to 96 nm over the same time period. DLS measurement of the sample after 8 h of irradiation was unable to reveal any meaningful information regarding the count rate and size of the particles due to the very low count rate of the sample. No precipitation of material was observed. It has been demonstrated that a decrease in the count rate (intensity of the scattered light) can stem from three factors: 1) a decrease in the total concentration of scattering particles in the system, 2) a decrease in the size of the particles in the system, and 3) a combination of the former two factors.[36] Taking into account the large decrease in count rate and relatively modest decrease in size, these results suggest that as the photodegradation process occurs, the dendrimersomes likely first undergo conversion into smaller assemblies such as micellar nanoaggregates whose diameters are

still measurable by DLS, but the number of these aggregates decreases continuously throughout the experiment as the dendrimers are completely converted into small molecule byproducts that do not significantly scatter light. TEM imaging after 8 h showed that no dendrimersomes remained and only a small degree of aggregate material was observed on the grid (ESI).

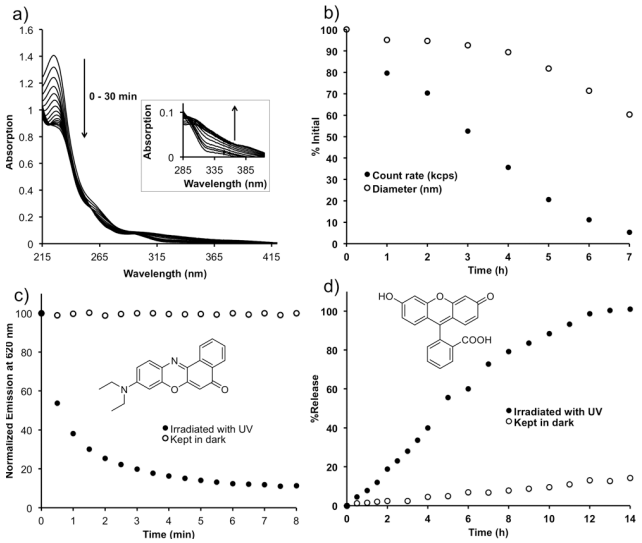


Fig. 2 a) UV-visible spectra of **7** in THF following irradiation with UV light over a period of 30 min; b) Photodegradation of dendrimersomes from **7** as measured by DLS count rate and diameter; c) Normalized fluorescent emission intensity of Nile Red-load dendrimersomes for samples exposed to UV light compared to those kept in dark (excitation at 540 nm and emission at 620 nm); d) Release profile of fluorescein for fluorescein-loaded dendrimersomes exposed to UV light compared to those kept in dark (excitation at 494 nm and maximum emission at 510 nm).

Finally, the potential for the photodegradation to trigger the release of both hydrophobic and hydrophilic cargo was investigated. The dye molecule nile red was selected as the hydrophobic cargo as its photostability when irradiated with UV light is well-documented.[44-46] In addition, nile red is strongly fluorescent in hydrophobic environments such as membranes, but is very poorly soluble in water, resulting in fluorescence quenching. This enables its release to be directly probed. To encapsulate nile red into the hydrophobic membranes of the dendrimersomes, this dye (2 wt% relative to **7**) was first dissolved in the DMSO solution of **7**. Water was then added dropwise to induce self-assembly, and dialysis against water was performed to remove DMSO. To study the photo-triggered release of nile red from dendrimersomes, the sample was placed in a quartz cuvette and irradiated with UV light. Fluorescence spectra were obtained at one min intervals. As shown in Fig. 2c, over a period of only 8 min, the fluorescence intensity of nile red decreased to ~10% of its initial value, consistent with its release from the membrane. This rapid rate of nile red release in comparison to the much longer time scale of the DLS study above suggests that only a small perturbation of the membrane is required to trigger release, whereas complete degradation to non-scattering species takes much longer. A control sample kept in the dark exhibited no change in the fluorescence intensity of nile red over the same time period, confirming that the release was indeed triggered by UV light.

Fluorescein was selected as a model payload to investigate the capability of the dendrimersomes to encapsulate and release hydrophilic molecules. This dye has been previously employed by other groups for UV-triggered release studies from polymersomes and shows a reasonable stability upon exposure to UV light.[36] In a control experiment, a 20% reduction in the fluorescence intensity of fluorescein-loaded dendrimersomes was observed upon irradiation with UV light for 10 min (ESI). However, this was not problematic as the sample was still quite fluorescent and the experiment was designed to monitor the fluorescence of released dye rather than a decrease in the fluorescence of encapsulated dye. To encapsulate fluorescein in the aqueous cores of the dendrimersomes, 0.15 mM fluorescein was dissolved in the water that was added to the DMSO solution of **7** during dendrimersome preparation. The resulting sample was stirred in the dark for 24 h to equilibrate and then was dialyzed against water for 9 h to remove any non-encapsulated fluorescein. A control dialysis experiment was performed to ensure that free dye at the same concentration was able to completely diffuse across the dialysis membrane in 9 h.

To study the light-triggered release of fluorescein from the dendrimersomes, the sample was irradiated with UV light for 10 min in a quartz cuvette. It was then transferred to a dialysis membrane and dialyzed against a large volume of water. The fluorescence intensity of dialysate was then measured at given time points. After each measurement, the sample was transferred back to the dialysate. As shown in Fig. 2d, the fluorescence intensity of the dialysate reached a plateau at ~14 h. It should be noted that the longer time scale required for the release of fluorescein in comparison with nile red can largely be attributed to the requirement for the dye to not only be released from the dendrimersome but also to diffuse across the dialysis membrane. To ensure that all the dye molecules had been released at 14 h, the fluorescence intensity of the sample within the dialysis membrane was directly measured. It showed no significant emission, confirming that fluorescein has been completely released. A control experiment was also performed with non-irradiated dendrimersomes. As shown in Fig. 2d, only 14% of the fluorescein was released over 14 h, likely via slow diffusion of the dye across the intact membrane. Therefore, this experiment confirmed the ability of UV light to also trigger the rapid release of hydrophilic cargo.

Conclusions

Amphiphilic Janus dendrimers composed of TEG-functionalized gallic acid as the hydrophilic block and G1 through G3 photodegradable dendrons were synthesized and their self-assembly in water was studied. While the G1 and G2 systems formed solid aggregates, the G3 system formed dendrimersomes under a number of different self-assembly conditions. Photodegradation of the dendrimersomes was studied by UV-visible spectroscopy, DLS and TEM, which confirmed their disintegration. In addition, they were demonstrated to exhibit triggered release of both hydrophilic and hydrophobic payloads upon irradiation with UV light. These results suggest the promise of these systems for controlled encapsulation and release applications.

Notes and references

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† Electronic Supplementary Information (ESI) available: detailed experimental procedures, NMR spectra for **3**-**7**, additional TEM images, additional DLS traces, UV-visible and fluorescence emission spectra. See DOI: 10.1039/b000000x/

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